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SHORT COMMUNICATION

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FIRST RECORD OF THE DIADEM LEAF-NOSED BAT *HIPPOSIDEROS DIADEMA* (E. GEOFFROY, 1813) (CHIROPTERA: HIPPOSIDERIDAE) FROM THE ANDAMAN ISLANDS, INDIA WITH THE POSSIBLE OCCURRENCE OF A HITHERTO UNREPORTED SUBSPECIES

OPEN ACCESS



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Abstract: The Diadem Leaf-nosed Bat *Hipposideros diadema* (E. Geoffroy, 1813) is recorded for the first time from the Andaman Islands, India. A solitary female specimen was collected on October 13, 2015 from a limestone cave on Baratang Island. Cranial measurements and other morphological characters indicate that the specimen differs from the endemic subspecies, the Nicobar Diadem Leaf-nosed Bat *Hipposideros diadema nicobarensis* (Dobson, 1871), but compares favourably with the South-east Asian subspecies, Mason's Diadem Leaf-nosed Bat *Hipposideros diadema masoni* (Dobson, 1872), in description, craniodental characters, and echolocation calls. This is the first record of *Hipposideros diadema* (E. Geoffroy, 1813) from Andaman Islands, and the subspecies Mason's Diadem Leaf-nosed Bat *Hipposideros diadema masoni* (Dobson, 1872) from India.

Keywords: *Hipposideros diadema masoni*, *Hipposideros diadema nicobarensis*, new record, range extension.

The Andaman & Nicobar Island archipelago (11.7401°N & 92.6586°E) is a highly species-rich group of islands in the Bay of Bengal, extending from the coast of southern Myanmar to Sumatra. The Andaman Islands comprise about 500 islands and islets, separated by a 'Ten-degree Channel' from the Nicobar Islands, which consist of around 20 islands. These two island groups hold a completely distinct type of rainforest ecosystem to that of mainland India, and also differ from each other (Balakrishnan 1989; FSI 2015). The Andaman Islands are part of the Indo-Burma biodiversity hotspot and the Nicobar Islands are part of the Sundaland biodiversity

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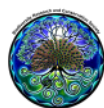
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hotspot (Myers et al. 2000). These islands are very rich in their fauna and flora, and a great number of taxa are endemic (Rao et al. 2013; Murugan et al. 2016).

The chiropteran fauna of the Andaman & Nicobar Islands has been summarized by Hill (1967), and more recently by Aul et al. (2014). Prior to the work of Aul et al. (2014), it was noted that species of the *Hipposideros* genus are restricted to the Nicobar Islands, and those of genus *Rhinolophus* to the Andaman Islands. Historically, as of 1967, one endemic subspecies — *Hipposideros diadema nicobarensis* (Dobson, 1871) was reported from Trinkut Island and Great Nicobar Island in the Nicobar group of islands (Hill 1967). This subspecies was also reported from Katchal Island (Aul & Marimuthu 2006). Aul et al. (2014) summarized the chiropteran diversity of the archipelago with a note on the effects of the 2004 Indian Ocean Tsunami, and also reported the presence of two species of genus *Hipposideros* — *H. fulvus* and *H. larvatus*, from the Andaman Islands. In this paper, we report for the first time, the presence of the Diadem Leaf-nosed Bat *Hipposideros diadema* from the Andaman Islands and provide the first specimen-based record of *Hipposideros diadema masoni* from India.

STUDY AREA AND METHODS

The authors have been surveying the Andaman Island group to document its chiropteran diversity since 2012. In 2012, a short-term survey was conducted on Baratang Island (12°6'21"N & 92°45'46"E), and subsequently a survey of most major islands was conducted from 2014–2016. During the initial survey on Baratang Island, the first author (BS) sighted a large hipposiderid bat with characteristic pale hairs on the flanks living amongst a colony of rhinolophids and myotis in a limestone cave. After this, on two occasions the same was sighted by us in 2014 and 2015. In October 2015, we collected this individual using a hoop net, took external morphometric measurements using vernier calipers (Mitutoyo LC=0.01) and made ultrasonic recordings using an Anabat SD1 (Titley Electronics). The bat escaped during transfer to a collection bag. Following this, about a fortnight later, we were alerted by some locals attempting to catch bats in the same cave. By the time we arrived on location, they had shot (using an air-rifle) a bat (Image 1), which on closer observation was identified to be the large hipposiderid bat that was caught by us before. The air-rifle pellet had, unfortunately, damaged the skull. We collected the specimen, preserved it as a voucher and deposited it in the Natural History Museum, Osmania University, Hyderabad, India. We also studied and compared the Andaman specimen collected during the present survey

with the specimens of large-bodied hipposiderids such as *Hipposideros diadema* (*H. d. masoni*: ZSI-K 291/126L, HZM 14.38116; *H. d. nicobarensis*: ZSI-K 10308, HZM 7.35313) and *Hipposideros larvatus* (ZSI-K 18554, ZSI-K 10290; HZM 17.34708; HZM 31.37787) at the Zoological Survey of India, Kolkata (ZSI-K), West Bengal, India and Harrison Institute (HZM), Sevenoaks, United Kingdom. The following external and craniodental measurements of the specimen were taken (all in mm): External: FA - forearm length, E - ear length, TL - tail length, Tib - tibia length, Hf - hindfoot length, 3mt - third metacarpal, 4mt - fourth metacarpal, 5mt - fifth metacarpal, 1ph3mt - first phalanx of third metacarpal, 2ph3mt - second phalanx of third metacarpal, 1ph4mt - first phalanx of fourth metacarpal, 2ph4mt - second phalanx of fourth metacarpal, Craniodental, GTL - greatest length of the skull, CBL - condylobasal length, CCL - condylocanine length, CM³ - maxillary toothrow, C¹-C¹ - anterior palatal width, M³-M³ - posterior palatal width, ZB - zygomatic breadth, BB - braincase breadth, CM₃ - mandibular toothrow, M - mandible length.

Genetic analysis—A 3mm wing punch was taken from the voucher specimen (AN2) and stored in silica gel. Genomic DNA was extracted using a QIAGEN Blood and Tissue kit (QIAGEN International, Netherlands). Polymerase Chain Reaction was carried out and we amplified a portion (~652 bp) of the mitochondrial cytochrome C oxidase subunit I gene using the primer pair VFi_t1 (5'-TGTA AACGACGGCCAGTTCTCAACCAACCACAAAGACATTG-3') and VR1_t1 (5'-CAGGAAACAGCTATG ACTAGACTTCTGGGTGGCCAAAGAATC-3') (Ivanova et al. 2007). The PCR reaction was performed in a 25µl reaction volume containing 2µl of template DNA, 12.5µl of 2X reaction buffer (0.05 U/µl Taq DNA polymerase, reaction buffer, 4 mM MgCl₂, 0.4 mM of each dNTPs), 0.5µl of each primer, and 9.5µl nuclease free water. The thermal profile followed included: 94°C for 1 min, five cycles of 94°C for 30 s, 50°C for 40 s, and 72°C for 1 min, followed by 35 cycles of 94°C for 30 s, 54°C for 40 s, and 72°C for 1 min, with a final extension at 72°C for 10 min (Ivanova et al. 2007). The amplified PCR products were sequenced using an ABI prism 3730 sequencer (Applied Biosystems, USA) and Big dye terminator sequencing kit (ABI Prism, USA). The gene sequence (Genbank accession number will be provided in revision) obtained was analyzed by using BLAST (Altschul et al. 1990) to determine similar sequences in the NCBI (www.ncbi.gov) database. Additional sequences of other related species were retrieved from the NCBI GenBank database for phylogenetic analysis. *Rhinolophus lepidus* was used as an outgroup taxon. The sequences were aligned



Image 1. Mason's Diadem Leaf-nosed Bat *H.d. masoni* from Baratang Island, Andaman Islands, India

using MUSCLE (Edgar 2004) incorporated in MEGA6 (Tamura et al. 2013) using default parameters. The alignment was processed in Gblocks v0.91b (Castresana 2000) and the final alignment of 581 bp was used for phylogenetic analysis. ModelTest (Posada & Crandall 1998) in conjunction with MEGA6 was used to choose the best fitting maximum likelihood DNA substitution model for the combined dataset, based on the corrected Bayesian Information Criterion (BICc) scores for each model type. The model chosen for the given data by ModelTest based on least BICc was TN93+I (Tamura-Nei + Invariant sites) (Tamura & Nei 1993). The chosen model for the combined dataset along with obtained likelihood parameters was used to generate a maximum likelihood (ML) tree through MEGA6. Bayesian analysis was carried out in BEAST v1.8.2 (Drummond et al. 2012) using default priors. The data were partitioned into ingroup and outgroup sets and the substitution model used was based on the results of ModelTest. The program was run for 10 million generations and tree sampling was made every 1000 generations. At the end of the analysis, the standard deviation of the split frequencies was below 0.01. Convergence was double checked using Tracer v1.6 (Rambaut et al 2014) to plot the likelihood scores against the generations. The first 25% of the total number of saved trees were discarded as burn-in and a majority consensus tree was made using TreeAnnotator, included in the BEAST v1.8.2 package.

RESULTS AND DISCUSSION

The specimen collected from the limestone cave on Baratang Island, on 13 October 2015, was a solitary adult female, sharing its roost with a large colony of bats comprising Andersen's Leaf-nosed Bat *Hipposideros pomona*, the Dawn Fruit Bat *Eonycteris spelaea*,

Horsfield's Myotis *Myotis horsfieldii*, the Andaman Intermediate Horseshoe Bat *Rhinolophus affinis andamanensis*, and a few individuals of the Andaman Horseshoe Bat *Rhinolophus cognatus*.

External measurements, morphology, and craniodental measurements taken suggested that the bat was the Diadem Leaf-nosed Bat *Hipposideros diadema* (E. Geoffroy, 1813). On enquiry with locals who regularly visited the caves as part of an ongoing project on monitoring Edible Nest Swiftlets, we were informed that there is a sizeable colony of the species in an inaccessible subterranean cave with a narrow opening. Acoustic monitoring using Anabat SD1 and Pettersson D500x (Pettersson Elektronik ab, Uppsala, Sweden) in the vicinity of the subterranean cave claimed by locals to host the bat colony did not yield any evidence of the presence of *Hipposideros diadema*.

Careful study of the morphology and craniodental features of the voucher specimen revealed that the *Hipposideros diadema* collected by us from the Baratang Island differs considerably in size and morphological characters from *Hipposideros diadema nicobarensis* (Dobson, 1871) - an endemic subspecies of Diadem Leaf-nosed Bat known from Nicobar Islands. The present specimen compares in description, craniodental characters, and echolocation calls with the Southeast Asian subspecies *Hipposideros diadema masoni* (Dobson, 1872).

The specimen collected by us differs from the *H.d. nicobarensis* in having a substantially longer forearm length (91.55mm vs. 66.06mm in *H.d. nicobarensis*) and a much larger skull (GTL: 30.09mm vs. 26.04mm) (Table 1). The Andaman specimen is slightly larger than *H. d. masoni* from Mawlamyine, Myanmar (type locality) and Lesser Sunda, Indonesia (Table 1), with respect to forearm length (FA), tibia length (Tib), third metacarpal length (3mt), fourth metacarpal length (4mt), condylocanine length (CCL), anterior palatal width (C^1-C^1), posterior palatal width (M^3-M^3), and braincase breadth (BB). However, its external measurements fall in the range of that of *H. diadema* from Cambodia (Table 1).

A brief description of the Andaman specimen of *H.d. masoni* is provided below:

External characters - A large bat with a forearm length of 91.55mm. Wing attached to the base of the outer toe, and the interfemoral membrane attached to the base of the inner toe. Ear broad at the base, with 10 ridges and a broadly rounded off tip; outer margin slightly concave beneath the tip. Noseleaf broad (14.1 mm), covering the whole muzzle. Anterior leaf (9.24mm wide) with no median emargination; internarial septum

Table 1. External and craniodental measurements of *H.d. masoni* from Baratang Island (Andaman Islands, India), Myanmar and Indonesia and *H.d. nicobarensis* from Great Nicobar Island, Nicobar Islands, India. Measurements are in mm. Abbreviations as stated in Study Area and Methods, † After Kitchener et al. (1992), § After Ith et al. (2011), - Data not available; * approximate measurement as skull is broken; ** premaxilla missing

Taxon	<i>H.d. masoni</i>	<i>H.d. masoni</i>	<i>H.d. masoni</i> †	<i>H. diadema</i> §	<i>H.d. nicobarensis</i>
Location	Baratang Island, Andaman Islands, India	Mawlamyine, Myanmar	Lesser Sunda, Indonesia	Cambodia	Great Nicobar Island, Nicobar Islands, India
Catalogue No.	AN2	ZSI-K 291/126L	-	-	ZSI-K 10308
External					
FA	91.55	85.09	80.2–90.2	83.2–93.2	66.06
E	27.01	27.94	25.2–28.2	23.3–30.8	22.55
TI	51.28	–	–	44.3–57.2	35.78
Tib	34.41	34.29	29.7–33.1	33.5–37.8	25.42
Hf	13.67	–	–	15.1–18.1	11.31
3mt	70.12	–	58.8–66.9	-	48.64
4mt	67.47	–	57.3–66.0	-	49.59
5mt	59.26	–	53.7–60.7	-	44.75
1ph3mt	31.36	–	25.0–30.9	-	23.26
2ph3mt	30.05	–	–	-	21.83
1ph4mt	23.26	–	–	-	17.55
2ph4mt	15.72	–	–	-	9.17
Craniodental					
GTL	30.21	28.41	28.5–30.5	31.04–33.08	26.04**
CBL	27.40	–	–	27.81–29.74	–
CCL	27.51	–	26.0–27.0	27.41–28.83	23.10
CM ³	12.20*	12.21	11.2–11.7	12.46–12.96	9.49
C ¹ –C ¹	7.97	7.76	7.0–7.8	8.04–8.47	6.64
M ³ –M ³	12.57*	12.33	11.6–11.9	12.15–12.83	9.82
ZB	17.21*	17.75	17.1–18.2	17.81–19.17	14.49
BB	13.79	11.08	11.0–12.3	-	11.12
CM ₃	13.76*	13.22	13.7–13.9	13.62–14.38	11.03
M	22.46	21.50	22.1–22.7	21.83–23.05	17.51

long, broad at the base and gradually tapering towards the tip, nostrils round in shape with well-developed narial lappets; intermediate leaf (8.25mm wide) is fleshy and with a median ridge; posterior leaf concave with the terminal portion folding back slightly and is erect, large (12.63mm wide) and fleshy with a single median vertical septum forming a small projection above the margin, and dividing the posterior leaf into two cells. A pair of glandular swellings is present behind the posterior leaf located above each eye on either side of the noseleaf. No frontal sac observed behind the posterior leaf. Three supplementary leaflets present. A bony projection observed on the undersurface of the chin. Hair on the back is cream coloured with greyish-brown terminal portion, hairs on the shoulders extending up to beneath the ears much darker, hairs on the flanks completely pale

cream in colour and form a continuous line on either side. Wing and interfemoral membranes uniform pale brown in colour. Hair on the face creamish coloured at the tips with pale brown bases. The ventral surface is pale in general. The noseleaf is yellowish-brown in colour, and is fleshy.

Craniodental - Skull large with a condylocanine length of 27.97mm. Sagittal and lambdoid crests well-developed with a shallow depression in the frontal region. Zygoma of each side robust, with well-developed dorsal process. Length of upper toothrow (CM³) 12.20mm; pm² minute in comparison to pm³ and canine, partially extruded from the toothrow, in contact with the base of the canine and pm³; pm³ and canine not in contact with each other. Two pairs of lower incisors, outer pair tricuspid and the inner pair bicuspid; incisors in contact with canine; pm₂ 25% of

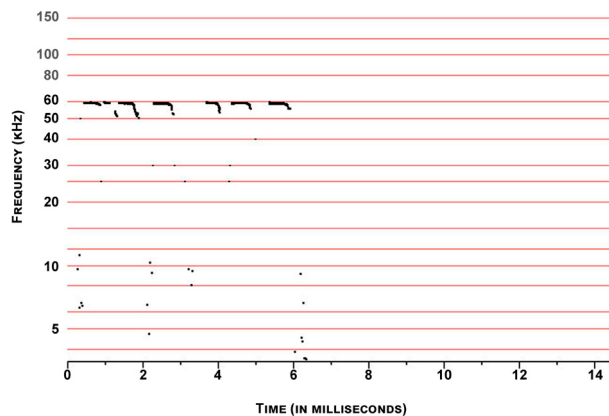


Image 2. Frequency–time graph of echolocation calls of Mason's Diadem Leaf-nosed Bat *H. d. masoni* from Baratang Island, Andaman Islands, India

the height of pm_3 . A small conical bony process observed on the symphysis of the mandible.

Echolocation calls - The echolocation calls of the species were recorded using an Anabat SD1 (Titely Electronics, Australia) bat detector. They were analysed in AnalookW software and frequency-time graphs were produced using a logarithmic scale of frequency (0–200 kHz). Each call was defined as an individual, discrete vocal pulse and the parameters measured were maximum frequency (Fmax), minimum frequency (Fmin), characteristic frequency (Fc) and duration (Dur). The calls produced by this species were of constant frequency (CF) with a short FM tail (Fig. 1). A total of 16 calls were recorded of which only five were of good quality and were analyzed further. The calls consisted of mean characteristic frequency (Fc) of 59.08 ± 0.24 kHz (range: 58.82–59.26 kHz), maximum frequency (Fmax) of 59.88 ± 0.25 kHz (range: 59.7–60.15 kHz), minimum frequency (Fmin) of 54.40 ± 1.97 kHz (range: 51.28–55.94 kHz) and duration of 4.77 ± 0.62 ms (range: 3.67–5.24 ms). The frequency characteristics of the echolocation calls of the specimen from the Andaman showed similarities with that of the South-east Asian forms, which produced FMAX in the range of 57.8–61.45 kHz (Phommexay 2009; Hughes et al. 2010).

Molecular analysis - Genetic analysis based on COI gene sequences indicated that the Andaman specimen of *Hipposideros diadema* is closely related to those from Laos and Vietnam, along with which it forms a distinct clade (Fig. 2) both in Maximum Likelihood and Bayesian Inference methods. The specimens from Malaysia form a separate clade. The phylogenetic position of *H. diadema* from Andaman is supported by high posterior probability (1.0) and bootstrap value (70.0). The relationship between other members of the species group needs to

be studied further to confirm the phylogenetic status of the *H. diadema* species group.

The echolocation calls, morphometrics (both external and cranio dental measurements) and the genetic analysis shows that the Andaman specimen matches those of the Southeast Asian specimens of *H. diadema*. The subspecies *H. d. masoni* (Dobson, 1872) is distributed throughout the Indomalayan region and differs from the typical *H. d. diadema* in possessing a single median septum in the posterior leaf dividing it into two cells (vs. a strong median septum with two weak lateral septa in *H. d. diadema*) (Andersen 1907; personal observations). The Andaman specimen matches *H. d. masoni* in possessing a single median septum dividing the posterior leaf into two cells, a small conical bony process from the symphysis of the mandible, and by having wings attached to the base of the outer toe (as opposed to wings attached to the base of the ankle in *H. d. diadema*). Interestingly, *H. d. nicobarensis* also shows similar characters as in *H. d. masoni* but is significantly smaller in overall external and craniodental measurements.

This is the first specimen-based record of *H. d. masoni* (Dobson, 1872) from Baratang Island and from the Andaman group of Islands, India. Our surveys in different localities of Little Andaman Island, Rutland Island, South Andaman Island, Middle Andaman Islands and islands of the Ritchie Archipelago did not yield sightings of this taxon. Even at the Baratang cave complex (consisting of 191 limestone caves) we detected this taxon only in one cave. The existence of the *H. d. masoni* (Dobson, 1872) in a cave on Baratang Island is enigmatic and requires further surveys to establish its continued presence north of 10° N latitude.

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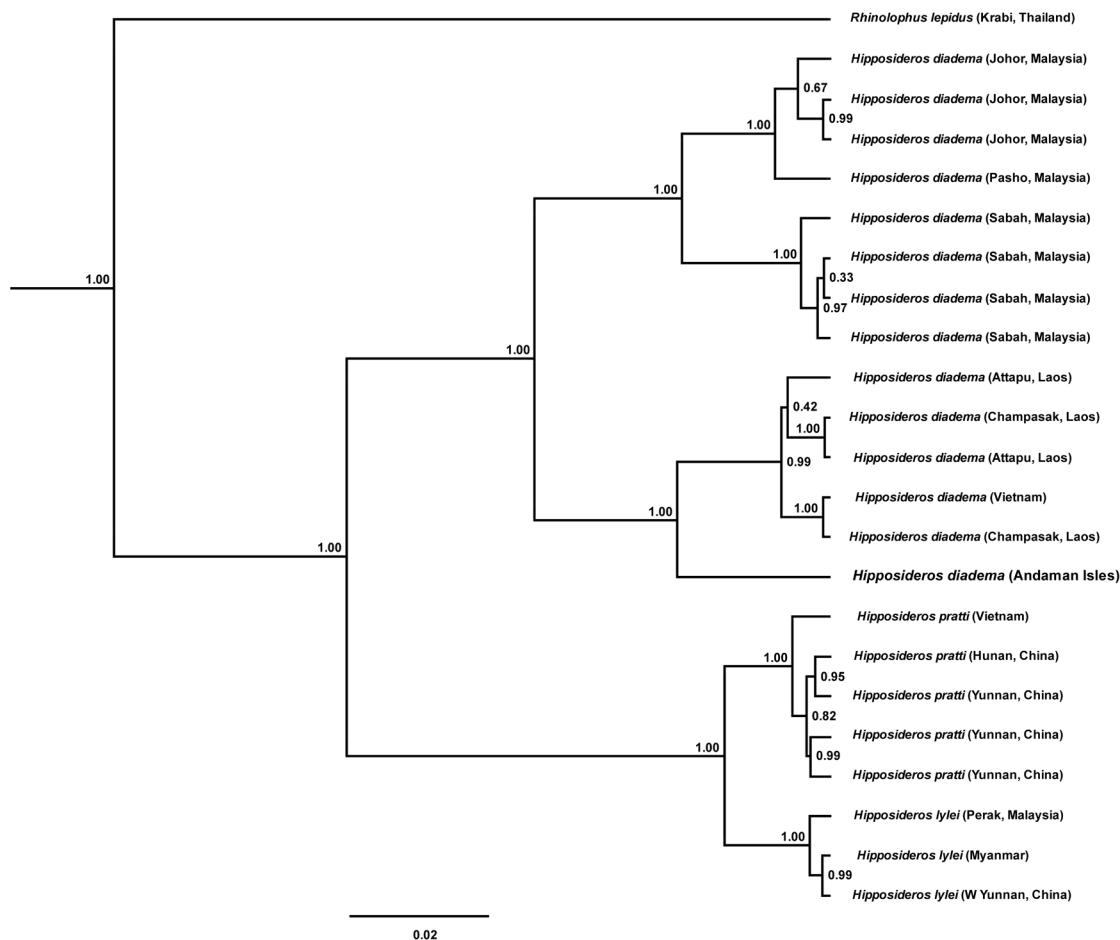


Image 3. Bayesian (BI) phylogenetic tree using 581bp portion of COI gene of mtDNA depicting the relationship of *H. diadema masoni* from Andaman Islands, India, with *H. diadema* from mainland and insular South-east Asia, and other members of the *H. diadema* species group. Values on nodes represent posterior probability.

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